

Is androconial secretion of *Pieris napi* (Lepidoptera, Pieridae) chemically dimorphic?

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Abstract Chemical analyses were conducted of male scent of *Pieris napi* collected in two districts, Yamaguchi Prefecture and Hokkaido. The androconial secretion of most individuals of Yamaguchi population comprised well-known compounds, linalool, nerol, neral and geranial, whereas some other males were found to secrete different and as yet unidentified monoterpenic components. We designate the former as Type I, and the latter, Type II. Further investigation on Hokkaido population revealed that it is of Type II. These findings are suggestive of the presence of at least two strains of *P. napi* of which male androconial secretion is chemically polymorphic.

Key words *Pieris napi*, androconial secretion, chemical dimorphism.

Introduction

Earlier work on chemicals involved in sexual communication between males and females of butterflies has documented that males of some species secrete and disseminate species-specific odoriferous compounds from the androconial organs or body tissues (Boppré, 1984; Honda, 1986). Such substances have been postulated to elicit a receptive response from conspecific females during the sequence of courtship, although not many publications have substantiated the pheromonal function of male secretions. Some male pierids endowed with androconia on their wings emit characteristic compounds; geranial and neral from *Pieris melete* (Bergström and Lundgren, 1973; Hayashi *et al.*, 1978; Kuwahara, 1979; Nakai, 1981), linalool, geranial and neral from *P. napi* (Hayashi *et al.*, 1978; Nakai, 1981, 1982), 13-methylheptacosane and esters of *n*-hexanol as sex pheromones from *Colias eurytheme* and *C. philodice*, respectively (Grula *et al.*, 1980), and a diversity of volatiles from both wings and body of *P. rapae* (Honda and Kawatoko, 1982) and *Delias* species (Ômura *et al.*, 2000). Recently, male odor of *P. melete* was shown to elicit leaning behavior from courted females (Kan and Hidaka, 1997), and methyl salicylate previously identified from the male body of *P. rapae* (Honda and Kawatoko, 1982) was demonstrated to be present also in the male body of *P. napi*, to be transferred to the female at copulation, and to act as an anti-aphrodisiac evoking mate-refusal posture from gravid females (Andersson *et al.*, 2000).

We have found that some males of *P. napi* emit several terpenic compounds other than so far known compounds from the butterfly. The present work briefly deals with chemical dimorphism of androconial secretion of *P. napi*.

Materials and methods

Insects

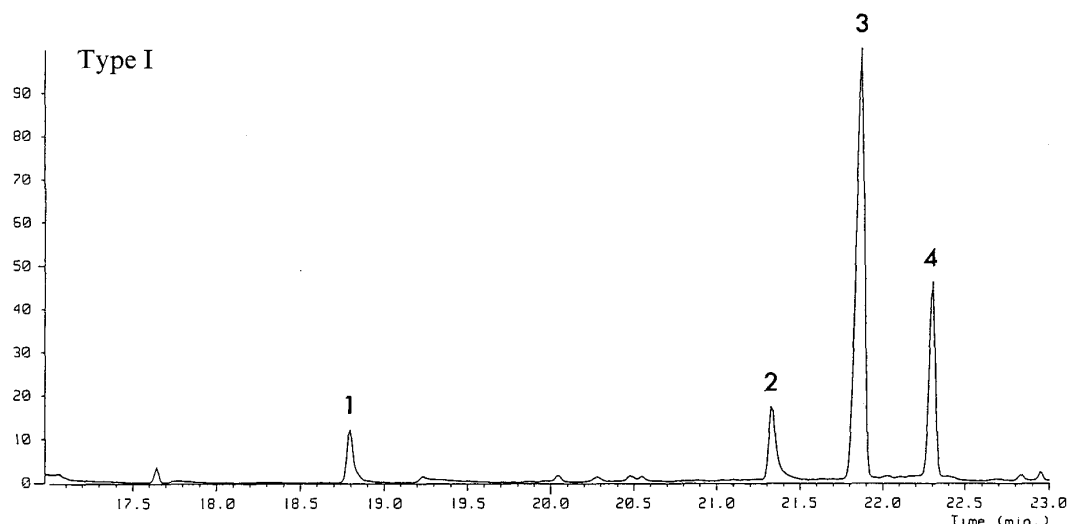


Fig. 1. Total ion chromatogram of volatiles from a male (Type I) of *Pieris napi* collected in Yamaguchi Prefecture. Peak 1: linalool, peak 2: nerol, peak 3: neral, peak 4: geranial.

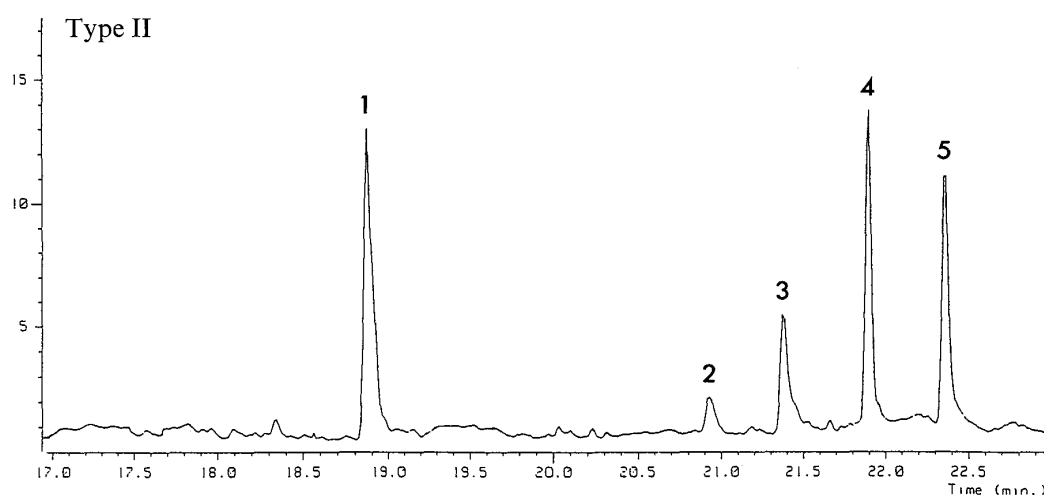


Fig. 2. Total ion chromatogram of volatiles from a male (Type II) of *Pieris napi* collected in Yamaguchi Prefecture.

Male adults of *P. napi* for chemical analyses were collected at Zohmeki, Atoh-cho, Yamaguchi Prefecture from early April to early October in 1998, and also at Kamifurano and Tokachishimizu, Hokkaido from July 20 to 22 in 2000. The Yamaguchi and Hokkaido populations may be regarded as *P. napi japonica* and *P. napi nesis*, respectively.

Collection and chemical analysis of volatile components

The scent substances emitted by a male were collected with Tenax TA (35–60 mesh, GL Sciences, Ltd). An individual lightly clamped by wire gauze was placed in a 300 ml separable flask, to which deodorized air (60 ml/min) was introduced. The air outlet was connected to a glass cartridge (3 mm I. D. × 160 mm) packed with 120 mg of Tenax TA. Air-entrained scent substances were continuously collected for 30 min at room temperature and those adsorbed on Tenax TA were desorbed at 250°C, trapped at –130°C, and introduced onto GC at 250°C through a TCT system (Chrompack CP-4020). Chemical

composition of volatile compounds was examined by gas chromatography-mass spectrometry (GC-MS). GC-MS analyses were carried out with a Hewlett Packard 5973 GC/MS system on a TC-1 fused-silica capillary column (0.25 mm I. D. \times 60 m). The injection temperature was 250°C, and the oven temperature was maintained at 40°C for the initial 5 minutes and then programmed from 40 to 280°C at a rate of 10°C/min. The flow rate of carrier gas (He) was 1 ml/min. EI-MS spectra were recorded at an ionization potential of 70 eV. Identification of components was based on a comparison of GC retention data and mass spectra with those of authentic chemicals.

Results and discussion

GC-MS analyses of volatile components revealed that most individuals collected in Yamaguchi emitted linalool, nerol, neral and geranial, all of which are compounds already reported from *P. napi* (Nakai, 1981, 1982). A typical total ion chromatogram (TIC) is shown in Fig. 1 and we designate such individuals that secrete well-known compounds as Type I hereafter. In marked contrast to Type I, some individuals of the same population were found to secrete quite different components (Type II), although their TIC (Fig. 2) was very similar. Peaks 1, 3, 4 and 5 in Fig. 2 exhibited the following characteristic base peaks at m/z 93, 93, 91 and 69, respectively. Although an attempt to identify these compounds was unsuccessful, they were all presumed to be monoterpenoids from their mass spectral fragmentation patterns.

On the other hand, three males collected in Hokkaido were all of Type II. Accordingly, the Yamaguchi population of *P. napi* is likely to consist of two strains that secrete chemically different androconial volatiles, viz. Type I and Type II. We have examined the secretion of 80 males of Yamaguchi population and found that about two thirds of them were Type I individuals, and the rest, Type II.

In the sulfur butterfly, *Colias eurytheme*, the male pheromone has been shown to be extraordinarily variable within populations in both the quantities and relative proportions of three major components (Sappington and Taylor, 1990a, 1990b). To the best of our knowledge, chemical dimorphism of the androconial secretion of *P. napi* has not hitherto been reported. At present, it is not clear whether Types I and II are distinct species, however, this finding may shed new light on the mating system of *Pieris* species in which chemically-mediated sexual communication is thought to be of great importance.

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摘 要

エゾスジグロシロチョウの発香器官分泌物には化学的な二型があるか? (中井 衛・本田計一・上田秀雄・大村 尚)

山口県と北海道で採集したエゾスジグロシロチョウ雄成虫の香気物質の化学分析を行った。山口個体群の大部分の個体の発香器官分泌物はよく知られたリナロール、ネロール、ネラールおよびゲラニールなどから構成されていたが、他の個体は化学構造の異なるモノテルペン系成分(未同定)を分泌することがわかった。そこで前者をⅠ型、後者をⅡ型と呼ぶことにする。一方、北海道個体群についても調べたところ、それらはⅡ型であることが判明した。これらの知見は、本種において雄の発香器官分泌物の化学的多型に基づく少なくとも2つの系統が存在することを示唆するものである。

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